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Association of allelic variants of thyroid-binding globulin with puberty in boars and responses to hemicastration☆

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ABSTRACT

Hemicastration of males increases weight of remaining testis when conducted before Sertoli cells cease to proliferate. The current studies re-examined responses to hemicastration in one-quarter Meishan crossbred boars that differed for two alleles of thyroid-binding globulin (TBG). In the first experiment, boars at 25 days of age with either allele did not differ in degree of testicular development; however, at 56 days, boars with the C allele had more advanced testicular development than littermates with the A allele as evidenced by testes with seminiferous tubules of larger diameter ($P < 0.008$) and greater weight ($P < 0.05$). At 10 months of age, boars hemicastrated at 25 days had a similar number of Sertoli cells in their single testis compared with both testes of control boars. However, in boars hemicastrated at 56 days number of Sertoli cells was less than the total number of Sertoli cells in the bilaterally intact controls; this reduction was greater ($P < 0.05$) in boars with the C allele than in those with the A allele. The second experiment confirmed earlier ($P < 0.05$) pubertal development in boars with the C allele relative to littermates with the A allele based on larger tubular diameter and the greater proportion of tubules with a distinct lumen at 60 and 80 days of age. These studies establish that boars with the C allele for TBG attain puberty at a younger age than those with the A allele thereby linking rate of pubertal development of boars with TBG or with gene(s) on the X chromosome in close proximity of TBG.

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1. Introduction

Hemicastration of immature males results in hypertrophy of the remaining testis with the magnitude of the response decreasing as they approach puberty (Cunningham et al., 1978). Number of Sertoli cells correlates positively with testicular weight and sperm production (Berndtson et al., 1987; Orth et al., 1988; Lunstra et al., 2003), and Sertoli cell proliferation increases after hemicastration of prepubertal but not adult males (Steinberger and Steinberger, 1971). In boars, hemicastration at or before 10 days of age produced a rapid compensatory response in the weight of the remaining testis (Clark et al., 1996) that persisted into early pubertal development (Kosco et al., 1987) and was associated with a rapid increase in number of Sertoli cells (Wells et al., 2008). Similarly, hemicastration at 1–2 months of age produced a near doubling in weight of the remaining testes (Sundby et al., 1981), but number of Sertoli cells increased (>2-fold) 2 months after hemicastration only in boars hemicastrated at 1 month of age (Putra and Blackshaw, 1985). In contrast to these studies with commercial crossbred boars, studies with one-half Meishan crossbred boars indicated that hemicastration at 1 or 10 days of age produced compensatory responses in testicular weight (Ford et al., 2001), but these changes were associated more with increases in Ser-

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toli cell size than with Sertoli cell numbers (Lunstra et al., 2003). Boars with small postpubertal testicular weight had no increase in number of Sertoli cells after hemicastration on 1 or 10 days of age; while boars with large postpubertal testicular weight had an increase in number of Sertoli cells of only 16% after hemicastration on 1 or 10 days of age. The report of Lunstra et al. (2003), was the only study noted that conducted postpubertal evaluation of Sertoli cells in hemicastrated boars. Thus, the possibility remains that prepubertal hemicastration of boars accelerates rate of Sertoli cell proliferation without producing a substantial increase in total number of Sertoli cells/testis within the postpubertal testis.

Subsequently, two allelic variants for thyroid-binding globulin (TBG), a gene that resides on the X chromosome, were established that associated with testicular size in Meishan crossbred boars (Nonneman et al., 2005). Boars with the C allele, which is inherited from the Meishan breed, had smaller testes than boars with the A allele, which is universal in commercial crossbred pigs. Furthermore, earlier pubertal development occurred in boars that matured with small testes compared with those that matured with large testes (Lunstra et al., 2003). The objectives of the current experiments were to investigate number of Sertoli cells and mass of seminiferous tubules after hemicastration of one-quarter Meishan crossbred boars and to examine the rate of pubertal development in boars that differed in TBG allele. These studies took advantage of females that were heterozygous for TBG to produce littermate boars that differed for the two alleles of TBG under investigation.

2. Materials and methods

2.1. Animals, management and sample collection

Boars that were used in these two experiments were from generations three and six of inter se mating of 3/4 commercial crossbred–1/4 Meishan pigs. This line was derived from the mating of boars and sows of a 1/2 Meishan–1/2 White composite resource population (Rohrer et al., 2001) with boars and sows of a Yorkshire–Landrace composite line. The dams that produced the experimental boars were heterozygous for the two alleles of TBG identified by Nonneman et al. (2005) providing the potential to produce littermates with each allele. Boars were reared at the U.S. Meat Animal Research Center using standard production and experimental practices that were in accordance with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Boars were weaned at 15–19 days of age, reared in confinement buildings and fed standard diets formulated to meet NRC requirements varying with increasing age to accommodate changing nutritional needs. Water was provided *ad libitum*. Body weights were obtained at birth, weaning, 56 days of age and at the age of postpubertal castration.

The right testis was removed in hemicastrated boars at the designated prepubertal ages ranging from 25 to 80 days of age. The remaining testis was removed at 10 months of age in the first experiment and at 8 months of age in the second experiment. For all castrations, boars were tranquilized with xylazine (2.5 mg/kg, i.m.; RX Veterinary Products, Westlake, TX) 15–20 min before administering thiopental sodium (Hospiara, Inc., Lake Forest, IL) i.v. followed with closed circuit anesthesia of isoflurane (Halcarbon Laboratories, North Augusta, SC) and oxygen.

2.2. Genotyping of TBG single nucleotide polymorphisms (SNP)

The two SNPs of TBG that were described by Nonneman et al. (2005) were genotyped using a primer extension assay on the Sequenom MassArray™ system (San Diego, CA, USA). Ten microliters PCR reactions contained 10 ng of genomic DNA, 0.25 U HotStar *Taq*, 1× of supplied buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, and 0.4 μM forward (5′-TCCGCCCAAGACAGAAGAGG-3′) and reverse (5′-AGGACAAAGAGTGCCAGAGC-3′) tailed primers. The primers were tailed with the 10 bp hME tail sequence (5′-ACGTTGGATG-3′). The primer extension reaction used 0.6 μM of probe primer (5′-CAGATGGAACAATACTAT-3′) and was performed according to the manufacturer's recommendations for hME chemistry (Sequenom, San Diego, CA, USA).

2.3. Experimental designs

In the first experiment, most boars within a litter were hemicastrated at 25 or 56 days of age with 1 or 2 boars/litter left intact. Within a litter, hemicastration was conducted on only one of the two dates. For the primary objective, the impact of the TBG allele and age at hemicastration, data were restricted to the litters with at least one boar of each allele resulting in 13 litters from 8 sires, 26 hemicastrated on day 25 (H25) and 21 hemicastrated on day 56 (H56). To estimate impact of hemicastration on number of Sertoli cells at 10 months of age, data were evaluated from all 16 litters produced by 10 sires, 25 bilaterally intact, 30 H25 and 27 H56.

In the second experiment, all boars within a litter were hemicastrated at one of the following ages: 30, 40, 50, 60 or 80 days. The influence of TBG allele on rate of testicular development was assessed in 15 litters that contained at least one boar of each allele, 70 boars produced by nine sires with four to 10 boars per allele at each age.

2.4. Testicular traits

After hemicastration at all prepubertal and postpubertal ages, testes were trimmed, weighed and split longitudinally. At 8 or 10 months of age, an approximate 10 g portion was frozen for subsequent evaluation of total daily sperm production (TDSP) by counting homogenization-resistant, elongated spermatid nuclei (Amann and Almquist, 1961; Ford et al., 2001). Additionally, two aliquots of tissue, <1 cm^3, were placed immediately into 4.0% paraformaldehyde (Sigma–Aldrich, St. Louis, MO) in 0.15 M PBS for approximately 5 h with gentle agitation followed by placement in fresh fixative for approximately 22 more hours; then dehydrated with increasing concentrations of ethanol

and imbedded in paraffin (McCoard et al., 2001; Lunstra et al., 2003).

Proportion of testis occupied by seminiferous tubules and diameter of seminiferous tubules were determined on 5 μm sections that were deparaffinized and stained with hematoxylin (Lunstra et al., 2003). The morphology of four random fields from one section of each aliquot of tissue per boar was evaluated via brightfield microscopy using computerized morphometric planimetry (Bioquant Nova Advanced Image Analysis 2000; R&M Biometrics, Inc.). Mass of the seminiferous tubules and interstitial tissue was estimated by multiplying testicular weight by 0.91 to adjust for weight of the tunica albuginea (Okwun et al., 1996), then multiplying by the appropriate proportion of the testis occupied by tubules or interstitial space.

For enumeration of Sertoli cells, 5 μm sections were deparaffinized, rehydrated, and stained for GATA-4 to specifically identify Sertoli cell nuclei (McCoard et al., 2001; Lunstra et al., 2003). The primary antisera were goat polyclonal (C-20; Santa Cruz Biotechnology, Santa Cruz, CA) raised against a peptide at the carboxy terminus of GATA-4 of mouse origin. Goat antisera were immunolocalized using the avidin–biotin immunoperoxidase system and Novared as the chromagen (Vectastain Elite ABC Kit; Vector Laboratories, Inc., Burlingame, CA). Non-immune sera or absence of the primary antisera were used to determine that nonspecific binding was not problematic.

2.5. Statistical analyses

Data were evaluated by Mixed Models procedures of SAS with TBG allele (A or C) and treatment (age at hemicastration) as main effects and with litter as a random effect (Littell et al., 1996). Means were compared using the PDIFF option when the *P*-value of $F < 0.05$ was detected. Body weight was evaluated separately for each age that the trait was recorded. For all traits evaluated on prepubertal testes, birth weight was a covariate, and for traits evaluated on the postpubertal testes, birth weight and final body weight were covariates. In Experiment 1, testes traits for each age group (25 days, 56 days and 10 months) were evaluated separately. In contrast, in Experiment 2 the prepubertal testicular traits for all five age groups (30, 40, 50, 60 and 80) were evaluated in the same analysis. Regression analyses were conducted with the regression procedure of SAS (1999) and were used to estimate the relationship of diameter of the seminiferous tubules within the testes removed at hemicastration (an estimate of pubertal development; Ford and Wise, 2009) with the weight of the remaining testis that was removed at either 8 or 10 months of age. Data are presented as mean ± SEM.

3. Results

3.1. Experiment 1: effect of TBG polymorphism on testicular traits after hemicastration

Birth weight of boars that were assigned to be unilaterally castrated was greater for those with the A allele of TBG than for littermates with the C allele ($P \leq 0.005$; 1.34 ± 0.05 kg vs. 1.19 ± 0.05 kg). Growth rate of boars with each allele was similar throughout the study, and at 10 months of age body weight was similar ($P > 0.2$; 144 ± 4.2 kg vs. 138 ± 3.8 kg).

All testicular traits increased from 25 to 56 days age (Table 1). At 25 days of age, testicular, tubular and interstitial weights were similar ($P > 0.6$) in boars with both alleles. In addition, percentage of the testis occupied with tubules and diameter of tubules were similar ($P > 0.3$). In contrast, at 56 days, boars with the C allele had greater ($P < 0.05$) tubular weight and greater percentage of testis occupied with seminiferous tubules that were larger in diameter ($P < 0.008$) than littermates with the A allele. These differences occurred in the presence of similar right testicular and interstitial weights ($P > 0.19$).

At 10 months of age, the 5 testicular traits that were evaluated differed with TBG allele (Table 2), but left testicular and interstitial weights of boars with the C allele and hemicastrated at 56 days were less ($P < 0.045$ and 0.011) than in the other three treatment groups. Number of Sertoli cells was less ($P < 0.004$) for boars with the C allele than those with the A allele, primarily reflecting fewer Sertoli cells in boars with the C allele that were hemicastrated at 56 days. Likewise, boars with the C allele had less ($P < 0.016$) TDSP than littermates with the A allele, and there was a trend ($P < 0.055$) for these two groups of boars to differ in weight of seminiferous tubules. The only trait affected by age at hemicastration was number of Sertoli cells ($P < 0.029$) with boars hemicastrated at 56 days having fewer Sertoli cells than those hemicastrated at 25 days.

Diameter of seminiferous tubules at hemicastration was related to the traits examined for boars hemicastrated at 56 but not at 25 days. In the 26 boars of the two allelic groups that were hemicastrated at 25 days, mean diameter of seminiferous tubules of the first testis was not correlated ($P \geq 0.11$) with testis weight, tubular weight, number

Table 1
Testicular traits at 25 or 56 days of age in littermate boars that differ in TBG allele[a].

Prepubertal testicular data	Hemicastration at 25 days			Hemicastration at 56 days		
	C[a]	A[a]	*P*-Value	C[a]	A[a]	*P*-Value
No. of boars	12	14		9	12	
Right testis weight (g)	3.0 ± 0.22	3.1 ± 0.21	0.7	9.4 ± 0.82	8.6 ± 0.80	0.19
Percent of tubules within the testis	30.6 ± 2.18	28.6 ± 1.92	0.3	44.5 ± 2.34	40.3 ± 2.26	0.05
Tubular mass (g)	0.9 ± 0.05	0.9 ± 0.04	0.9	4.2 ± 0.38	3.4 ± 0.35	0.05
Interstitial mass (g)	2.1± 0.21	2.2 ± 0.20	0.6	5.2 ± 0.54	5.2 ± 0.53	0.9
Tubular diameter (μm)	59.9 ± 2.59	59.5 ± 2.46	0.8	69.6 ± 3.05	58.0 ± 2.76	0.008

[a] Thyroid-binding globulin (TBG) alleles; C: Meishan allele of TBG; A: crossbred allele of TBG.

Table 2
Testicular traits at 10 months of age in littermate, hemicastrated boars that differ in TBG allele[a].

Ten months testicular data	Hemicastration at 25 days		Hemicastration at 56 days		*P*-Values		
	C[a]	A[a]	C[a]	A[a]	TBG allele	Age	Allele × age
No. of boars	12	14	9	12	–	–	–
Left testis weight (g)	434 ± 32[a]	456 ± 30[a]	324 ± 36[b]	485 ± 32[a]	0.012	0.21	0.045
Tubular mass (g)	222 ± 23[ab]	236 ± 22[b]	157 ± 26[a]	233 ± 23[b]	0.055	0.19	0.17
Sertoli cells ($\times 10^9$)	10.8 ± 1.37[a]	14.0 ± 1.29[a]	6.3 ± 1.53[b]	12.3 ± 1.34[a]	0.004	0.029	0.33
Interstitial mass (g)	174 ± 15[ab]	179 ± 15[ab]	138 ± 17[a]	216 ± 15[b]	0.006	0.97	0.011
TDSP[b] ($\times 10^9$)	7.3 ± 0.89[ab]	8.6 ± 0.84[b]	5.6 ± 0.99[a]	9.1 ± 0.87[b]	0.016	0.55	0.25

Means within a row that do not share a common letters (a–b) differ ($P < 0.05$).
[a] Thyroid-binding globulin (TBG) alleles; C: Meishan allele of TBG; A: crossbred allele of TBG.
[b] Total daily sperm production.

of Sertoli cells, interstitial weight or TDSP at 10 months of age. In contrast, in the 21 boars that were hemicastrated at 56 days, mean diameter of the seminiferous tubules of the first testes was correlated negatively with testicular weight ($P < 0.002$), tubular weight ($P < 0.002$), number of Sertoli cells ($P < 0.001$), interstitial weight ($P < 0.01$) and TDSP ($P < 0.002$) at 10 months, $r^2 = 0.66, 0.67, 0.74, 0.57$, and 0.67, respectively.

To estimate the Sertoli cell response to hemicastration, testicular traits were evaluated in all 82 boars assigned to the study. In boars hemicastrated at 25 days of age, number of Sertoli cells within their single testis was similar ($P > 0.43$) to the number of Sertoli cells in both testes of intact boars (Fig. 1A). In contrast, hemicastration at 56 days of age reduced the number of Sertoli cells compared with the total number in intact boars ($P < 0.007$); the reduction in H25 boars relative to H56 boars approached significance ($P < 0.059$; Fig. 1A). Total testicular weight (557 ± 25, 433 ± 25 and 406 ± 26 g for intact, H25 and H56 boars, respectively), weight of seminiferous tubules (Fig. 1B) and TDSP (Fig. 1C) were all reduced ($P < 0.001$) in both groups of hemicastrated boars. Among the three groups of boars, TDSP/g of seminiferous tubule was similar ($3.9 \times 10^7 \pm 0.37$; $P > 0.56$).

The influence of the TBG allele within the 82 boars was consistent with the results reported in Table 2. Boars with the A allele had more Sertoli cells at 10 months of age compared with those with the C allele ($P < 0.001$; 1.41-fold greater) concurrent with their greater testicular ($P < 0.001$; 1.22-fold) and tubular ($P < 0.01$; 1.23-fold greater) weights and TDSP ($P < 0.01$; 1.34-fold greater).

3.2. Experiment 2: prepubertal pattern of testicular development

Birth weight of the 70 boars was similar among those assigned to the five ages of castration but tended to be smaller ($P < 0.105$) for boars with the C allele than those with the A allele of TBG (1.30 ± 0.05 kg vs. 1.22 ± 0.05 kg). Body weight at castration at 8 months was similar in these two groups, 126 ± 5 kg vs. 125 ± 3 kg for boars with the A and C alleles.

Testicular weight, weight of seminiferous tubules, percentage of the testis occupied by tubules, diameter of tubules and percentage of tubules with a distinct lumen all increased ($P < 0.001$) with age from 30 to 80 days. Boars with the C allele had greater testicular weights at 60 and 80 days of age (Fig. 2A) than boars with the A allele creating an interaction of age with TBG allele ($P < 0.009$). Across all days, percentage of testis occupied by tubules was greater ($P < 0.035$) in boars with the C allele than in those with the A allele; the greatest difference was at 60 days ($46 \pm 2\%$ vs. $33 \pm 3\%$). Likewise tubular diameter was greater ($P < 0.001$) across all days in boars with the C allele than for those with the A allele (Fig. 2B). Concurrently, the

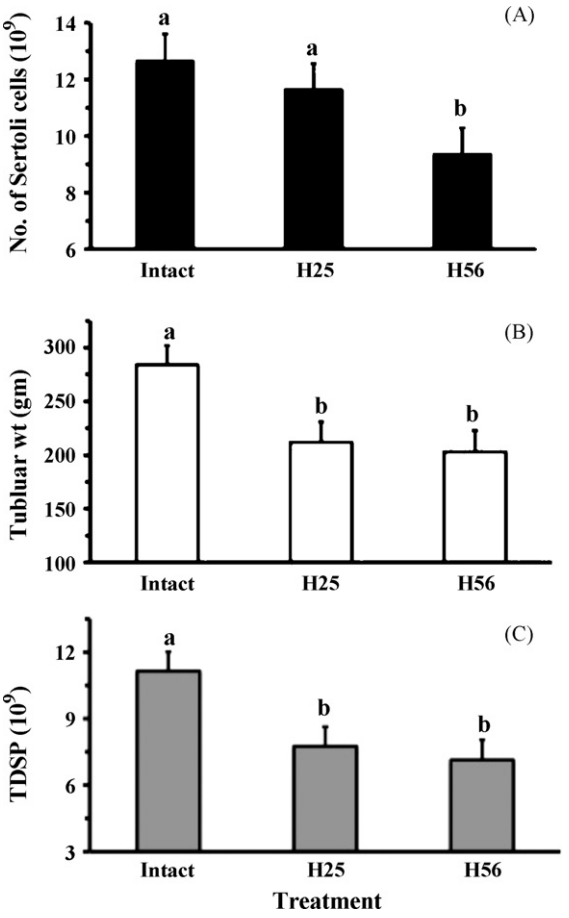

Fig. 1. Mean number of Sertoli cells (A), weight of seminiferous tubules (B) and total daily sperm production (TDSP) (C) in Meishan crossbred boars that were hemicastration at 25 or 56 days of age. Means that do not share a common superscript differ, $P < 0.01$ except (A) where $P < 0.059$ for H25 vs. H56.

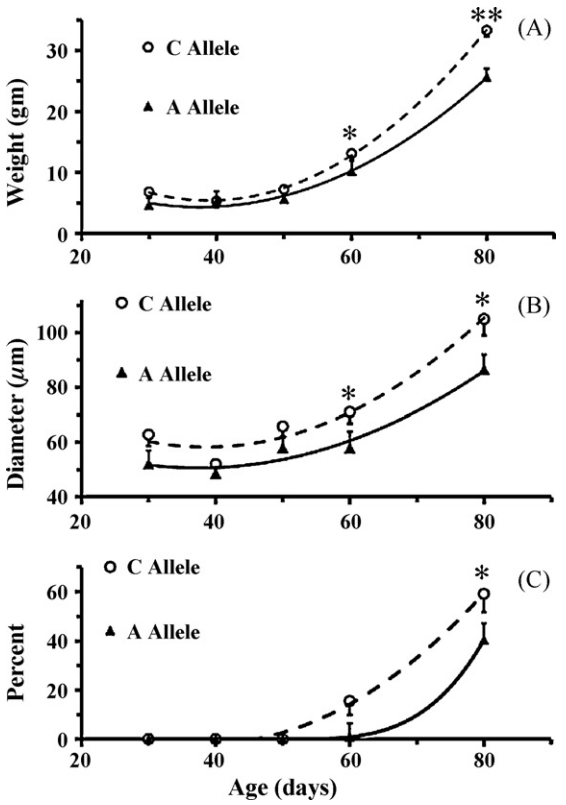

Fig. 2. Testicular traits during pubertal development in littermate, Meishan crossbred boars that differed in their allelic variant of thyroid-binding globulin. Changes associated with increasing age are presented for weight of the right testis (A), mean diameter of seminiferous tubules (B) and percentage of tubules with a distinct lumen (C). The C allele is from Meishan ancestry and the A allele is found in commercial crossbred in pigs. Means at designated ages differ, $^{*}P < 0.05$, $^{**}P < 0.01$.

percentage of tubules with a distinct lumen was greater ($P < 0.04$) for boars with the C allele than those with the A allele (Fig. 2C). Weight of seminiferous tubules was greater ($P < 0.001$) in boars with the C allele compared with littermates with the A allele only on days 60 and 80 (5.5 ± 1.9 g vs. 3.1 ± 1.9 g on day 60 and 17.4 ± 1.3 g vs. 11.4 ± 1.3 g on day 80).

At 8 months of age, testicular weights ($P < 0.001$) and weights of seminiferous tubules ($P < 0.002$) were less in boars with the C allele than in those with the A allele (340 ± 21 g vs. 453 ± 24 g for testicular weight and 208 ± 17 g vs. 292 ± 19 g for weight of tubules). Combined among boars with either allele, diameter of seminiferous tubules at days 30, 40 or 50 was not associated ($P > 0.28$) with testicular weight at 8 months, but diameter of seminiferous tubules at days 60 and 80 was negatively associated with subsequent weight of the testes at 8 months of age ($P < 0.03$, $r^2 = 0.22$). TDSP was less ($P < 0.001$) in boars with the C allele than in those with the A allele ($4.1 \pm 0.4 \times 10^9$ sperm per day vs. $5.9 \pm 0.5 \times 10^9$ sperm per day), but daily sperm production/g of seminiferous tubule was similar ($P > 0.50$) in these two groups ($2.0 \pm 0.16 \times 10^7$).

4. Discussion

The present study clearly documented earlier pubertal development in testes of boars with the C allele of TBG relative to littermate boars with the A allele. This was substantiated at 56 days of age when boars with the C allele had a greater proportion of seminiferous tubules with larger diameter and greater mass. Then, at older ages, boars with the C allele had an earlier appearance of a distinct lumen within their tubules. The C allele originated from the Meishan breed (Rohrer et al., 2001) and is found in other crossbred pigs that contain breeds of Chinese origin (Ponsuksili et al., 2005), but to date, it has not been observed in commercial crossbred pigs. The association of small testicular size with earlier puberty in Meishan crossbred boars was first proposed by Lunstra et al. (2003) and later associated with the allelic variants of TBG (Nonneman et al., 2005). This association of TBG allele has remained through eight generations since the initial crossing of Meishan with commercial crossbreds indicating that rate of pubertal development is regulated by TBG or by gene(s) in close proximity of TBG on the X chromosome. To date, physiological changes in secretion of thyroid hormones have not been implicated in modification of rate of pubertal development of boars (Tarn et al., 1998; Klobucar et al., 2003), but the A isoform of TBG has greater affinity for thyroxine than the C isoform (Nonneman et al., 2005).

Proliferation of Sertoli cells ceases with development of the Sertoli cell barrier associated with increased secretion of tubular fluids (Sharpe et al., 2003; França et al., 2005). The lack of any correlation of tubular diameter within the testis removed at 50 days of age or younger with weight of the remaining testis when evaluated after pubertal development supports the hypothesis that Sertoli cell proliferation increased in these younger boars. Consequently, the weight of the second testis reflected the genetic potential of the boar and not his stage of pubertal development. In contrast, the negative correlation of tubular diameter in the first testis that was removed with postpubertal weight of the second testis when hemicastration was conducted at 56 days of age or older supports arrested proliferation of Sertoli cells. The boars with larger diameter tubules had a reduction in their ability to produce a compensatory increase in testicular weight supporting recent observations in commercial crossbred boars (Ford and Wise, 2009). Although reduced testicular size and earlier puberty are correlated traits in Meishan crossbred boars, this relationship is not universal to all genetic lines of boars. Meishan and Piau boars both have smaller testes than commercial boars, but Meishan boars reach puberty at earlier ages (Harayama et al., 1991; Lunstra et al., 1997; Kanematsu et al., 2006) while Piau boars reach puberty at comparable or older ages (Castro et al., 1991; França et al., 2000) than commercial boars. Couple these observations with those of Harder et al. (1995) and Huang and Johnson (1996) who reported that direct selection for larger testes reduced age at puberty. Thus, in boars selected for larger testicular size, Sertoli cell proliferation occurred more rapidly before the onset of arrested proliferation of Sertoli cells than in boars of the control line thereby producing larger testes with greater sperm production. Consequently, testicular

size and pubertal age are not necessarily tightly coupled physiological events in boars.

In the current study, a definitive increase in number of Sertoli cells was observed following hemicastration of 25-day-old, one-quarter Meishan crossbred boars, and this increase was diminished in the 56-day-old boars. These findings agree with the earlier acute response reported by Putra and Blackshaw (1985) but differ from those of Lunstra et al. (2003). Seemingly, the one-half Meishan boars used by Lunstra et al. (2003) differed physiologically from one-quarter Meishan boars in their response to hemicastration. Clearly, the factors responsible for the failure of one-half Meishans to have a sizeable increase in number of Sertoli cells after hemicastration were diminished sufficiently in the one-quarter Meishan boars used in the current study such that the compensatory response to hemicastration was more similar to that of commercial boars. However, the increase in weight of testes and seminiferous tubules was compromised somewhat and failed to fully equal the mass of testes in bilaterally intact boars as observed by Sundby et al. (1981).

The factors that activate and drive the testicular compensatory response in boars are not understood. During the first 2 weeks of life, a sizable proportion of Sertoli cell nuclei in purebred Meishan boars (>15%) are positive for Ki67, indicative of proliferating cells (McCoard et al., 2003). Thus, a sufficient proportion of Sertoli cells of 1- and 10-day-old one-half Meishan boars would be in a proliferative state. Secretion of FSH increases after hemicastration of boars (Kosco et al., 1987; Clark et al., 1996; Ford et al., 2001) as does Sertoli cell proliferation (Wells et al., 2008). In rats, response to hemicastration is FSH-dependent (Brown et al., 1991), and neonatal Sertoli cells of pigs proliferate in response to FSH stimulation in vitro (Krantic and Benahmed, 2000). However, treatment of boars with FSH from 8 to 40 days of age relative to control boars stimulated only lumen development of the seminiferous tubules at 100 days without having significant impact upon other testicular traits (Swanlund et al., 1995). None of these FSH-treated boars were evaluated for postpubertal testicular traits. In a subsequent study, doubling plasma FSH concentrations in neonatal boars via exogenous supplementation failed to increase number of Sertoli cells, testicular size or sperm production at adulthood (Wagner and Claus, 2009) as occurs when neonatal rats are treated with FSH (Meachem et al., 1996). Contrary to the above findings, França et al. (2000) observed a strong correlation of increased FSH secretion in boars during periods of Sertoli cell proliferation. Thus, to date, a predominant, consistent role for FSH as a stimulus for Sertoli proliferation of boars and subsequent enhancement of sperm production lacks unwavering support.

Intra-testicular mechanisms may play a prominent role in the testicular compensatory response to hemicastration. Inhibition of testicular estrogen synthesis during neonatal development with a specific aromatase inhibitor increased number of Sertoli cells, testicular weight and sperm production (At-Taras et al., 2006a; Berger et al., 2008). These changes occurred with no observed changes in gonadotropin secretion (At-Taras et al., 2006b). These are the first reports of exogenous stimulation of Sertoli cell proliferation in prepubertal boars that produced an apparent permanent increase in postpubertal number of Sertoli cells associated with enhanced sperm production. Consequently, the increase in FSH that occurs after hemicastration may be secondary to other mechanisms for increasing number of Sertoli cells in boars hemicastrated at 25 days of age in the current study and testicular weight in the study of Sundby et al. (1981).

5. Conclusions

Boars with the C allele for TBG reached puberty at an earlier age than littermate boars with the A allele. Number of Sertoli cells in the single testis of boars hemicastrated at 25 days of age increased to the number observed in control boars with two testes, and this response was diminished in boars hemicastrated at 56 days of age with the response being less in boars with the C allele than in those with the A allele for TBG. Equal number of Sertoli cells in boars hemicastrated at 25 days of age did not increase testicular weight to that of total testicular weight of bilaterally intact control boars.

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